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SURFACE ULTRASTRUCTURE OF THE SCYPHOPOLYP, *CHRYSAORA QUINQUECIRRHA*

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Cnidarians are aquatic animals thought to represent the simplest and perhaps basic structural organization of multicellular, metazoan organisms. The body plan consists of a blind sac with a single opening, the mouth, surrounded by tentacles. The body wall consists of two cell layers; an outer epidermis and an inner gastrodermis separated by a gelatinous mesoglea of varying thickness and cellularity. The enclosed space is referred to as the gastrocoel or gastrovascular cavity. Water in the gastrocoel is occasionally flushed by periodic body contractions and, in many cases, continually by currents generated by ciliated gastrodermal cells. Cells of both epithelial layers thus have direct contact with the external environment.

In diploblastic organisms, functional specialization involves the differential distribution of various cell types, as well as structural specialization of the surface of individual cells. Despite the importance of cell interaction with the environment in cnidarian function, little consideration has been given to surface ultrastructure in contrast to the extensive literature dealing with various other aspects of the relatively few cnidarian cell types. Furthermore, most of these studies represent the classes Hydrozoa and Anthozoa. Therefore, this study was undertaken to characterize the surface features of a Scyphozoan polyp, *Chrysaora quinquecirrha* by scanning electron microscopy and, when possible, to relate such structure to function.

MATERIALS AND METHODS

The polyps used in this study were obtained from populations of *Chrysaora quinquecirrha* maintained in culture in the laboratory. Specimens were prepared for scanning electron microscopy (SEM) by fixation at room temperature in 5% glutaraldehyde made up with sea water (10‰). Polyps were rinsed in tap water for 30 minutes, dehydrated through a graded ethanol series to isoamylacetate, and dried by the critical point method with carbon dioxide. For observation of gastro-

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dermal structures, some dried polyps were sectioned longitudinally with microforceps and a razor blade. The animals were then affixed to specimen stubs with scotch (3M) transfer tape, coated with carbon and gold-palladium, and examined with an Etec Autoscan electron microscope operated at 45° tilt and at 20 kV unless otherwise indicated.

Observations on living specimens were made using bright field and phase contrast microscopy. Studies of currents generated by ciliary motion were facilitated by the addition of a dilute suspension of carmine particles. Gastrodermal currents were observed in animals which were cut in half lengthwise with a razor blade.

RESULTS

Chrysaora polyps are goblet-shaped organisms which attach to hard substrata by means of an aboral pedal disc (Fig. 1). A slender body region, the stalk, extends from this point of attachment and then widens abruptly to form the calyx. The oral disc (Fig. 2), with its centrally located mouth and surrounded by a single row of tentacles, forms the distal end.

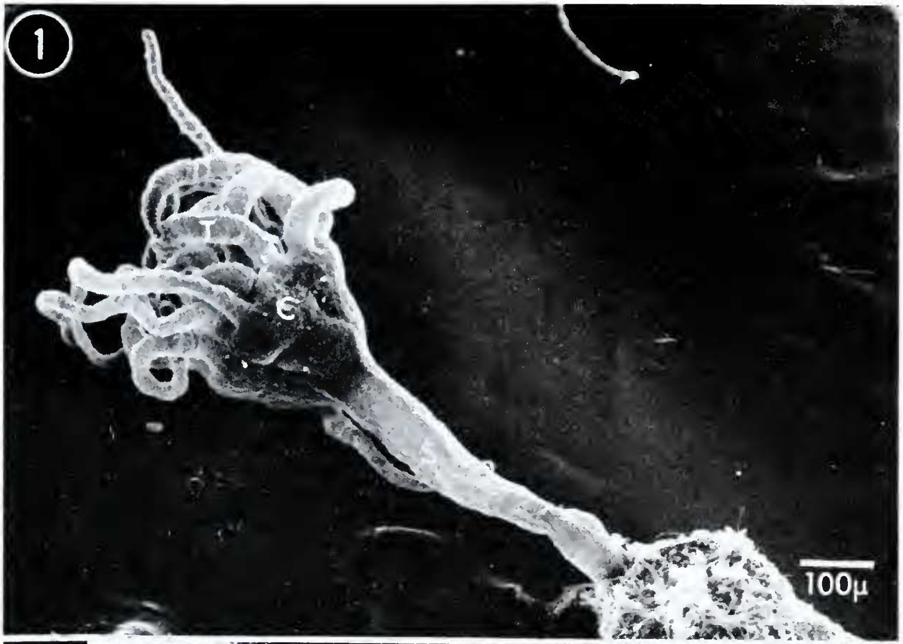
Epidermis

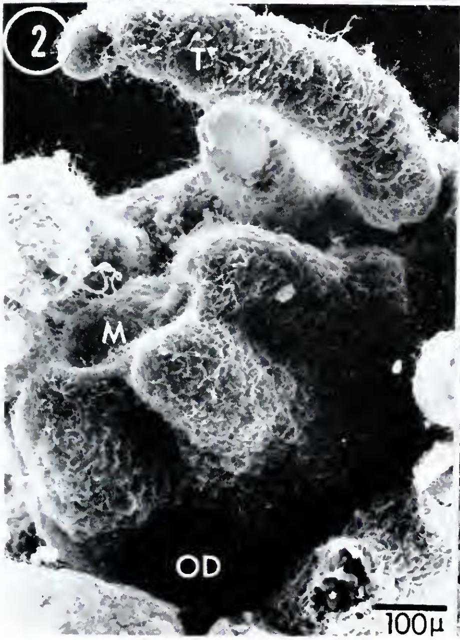
All regions of the epidermis are ciliated. A moderate number of cilia appear on the oral disc and epithelium between the tentacles (Figs. 2, 3). In contrast, the upper calyx is more heavily ciliated (Fig. 4) with the incidence of cilia decreasing toward the lower portion of the calyx (Fig. 5) and along the stalk toward the pedal disc.

Most cells possess a single cilium surrounded at the base by a circlet of small, closely spaced microvilli (Figs. 5, 6). The cilia on the oral disc arise from a crater formed by the fusion of 9–12 microvilli (Fig. 6) and are highly motile. Cilia of this nature are also common on the calyx and tentacles (Figs. 7, 8). In addition to those surrounding the cilia, epidermal cells of tentacles, calyx and stalk possess numerous, scattered microvilli (Figs. 5, 7). Fine, filamentous strands which occur between microvilli (Fig. 5) are thought to represent mucus preserved during fixation. There is a conspicuous paucity of microvilli on the oral disc (Figs. 3, 6).

Other cilia arise from a broad, conical base (Fig. 8) probably consisting of fused microvilli. These cilia are most abundant on the tentacles. They are present to a lesser extent on the calyx, and they are occasionally seen on the stalk. The distribution of these broad-based cilia corresponds to the distribution of nematocysts observed by light microscopy in living polyps. Discharged nematocyst threads originate in close proximity to the broad, conical bases (Figs. 9, 10) and thus these cilia are considered to be cnidocils. As viewed by phase microscopy in living animals, cnidocils appear as long, fairly straight structures which vibrate slightly in the current generated by the motile epithelial cilia.

Two types of nematocysts, microbasic euryteles and atrichous isorhizas (nomenclature according to Weill 1930, 1934), are abundant on the tentacles and to a lesser extent on the calyx. Atrichous isorhizas actually possess small, uniformly distributed barbs along the dischaged thread (Figs. 7, 9, 10). Tentacle nematocysts can be made to discharge when the polyp is placed in 0.5% acidified methylene





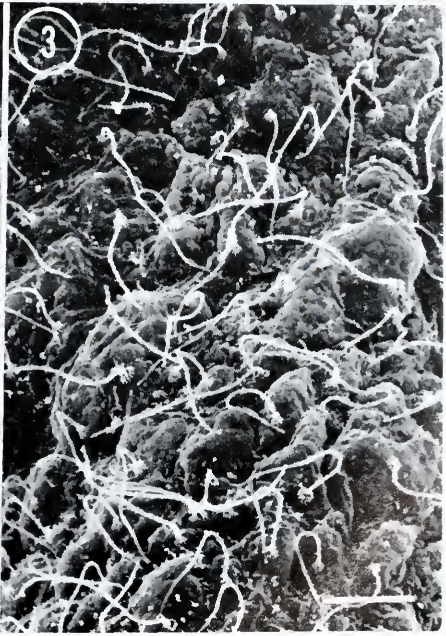
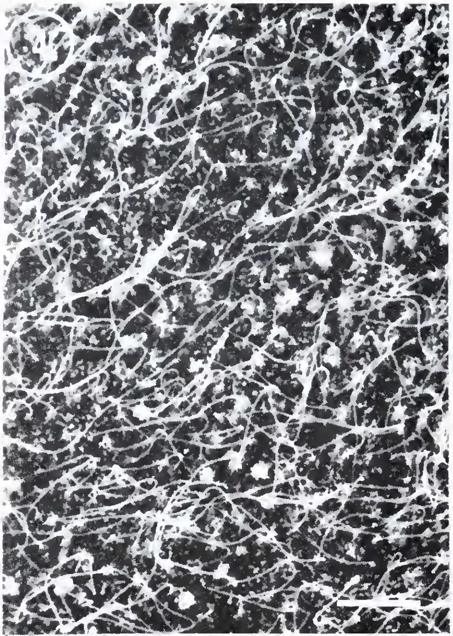
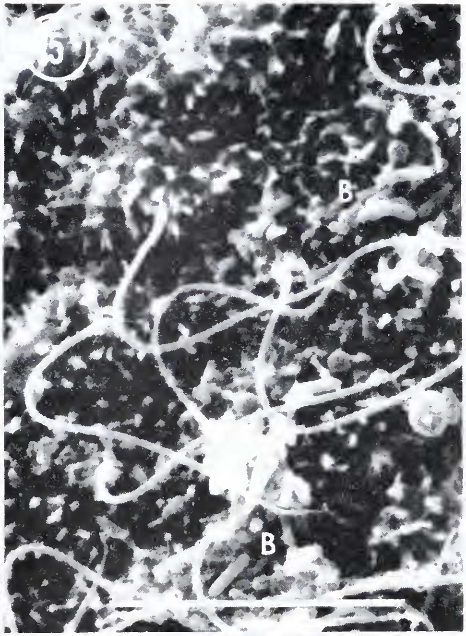


FIGURE 1. *Chrysaora* polyp illustrating the major body regions; tentacles (T), calyx (C) and stalk (S).

FIGURE 2. Oral disc (OD) revealed by removal of most surrounding tentacles (T). The mouth (M) is centered in a raised hypostome with four radial folds.

FIGURE 3. Oral disc showing sparse ciliation and few microvilli on the smooth cell surfaces. Compare with the heavily ciliated inner surface of the oral disc (Fig. 12); 10 μm scale marker.





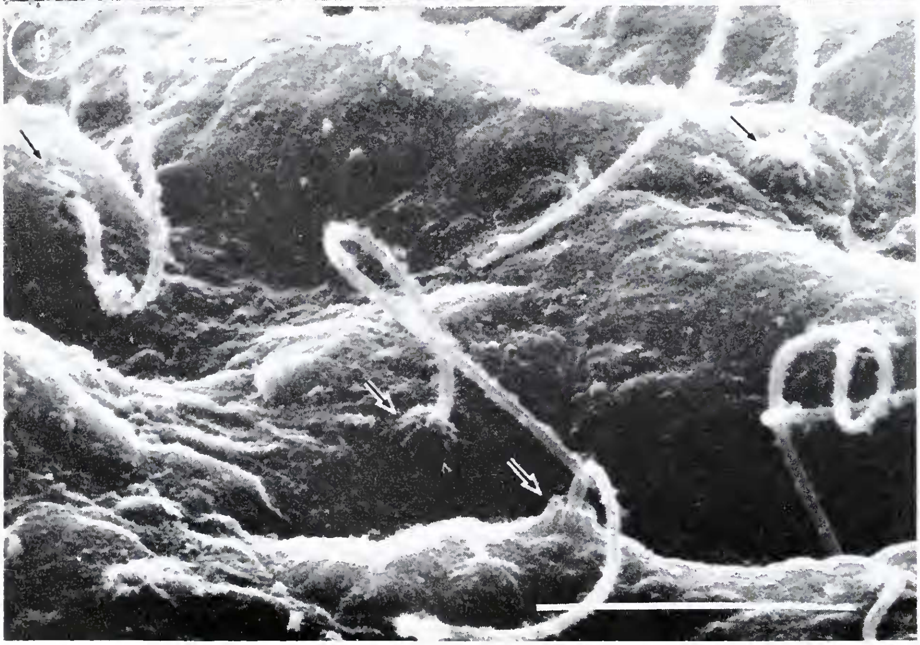


FIGURE 4. Heavily ciliated region of the upper calyx. Most cilia are curved in the same direction suggestive of ciliary movement and consistent with the rapid current observed in this area; 10 μm scale marker.

FIGURE 5. Surface of lower calyx with fewer cilia and numerous microvilli. Microvilli are loosely clustered around the base of each cilium. Note the numerous bacteria (B) adhering to

blue. Nematocysts in the calyx also discharge under these conditions and thus are probably functional on the body as well.

Gastrodermis

In scyphopolyps, the gastrocoel is divided by four septae which extend from the region of the oral disc to the junction of the calyx and stalk (Fig. 11). Of particular interest is the nature of the epithelium which lines the oral disc and the free edges of the septae. This epithelium has been referred to by Chapman (1966) as the scyphopharynx-filament complex (SPC) and its distinctive appearance in comparison to other regions of the polyp has long been recognized (Tcheou-Tai-Chuin, 1930).

The SPC is the most heavily ciliated region of the polyp (Figs. 12, 13). Most cilia arise from circlets of short, fused microvilli. Many cilia in this region, especially along the septal edge, are surrounded by rather long, fused microvilli (Fig. 14) which form broad conical bases like those which distinguish tentacle cnidocils. Nematocysts, mainly microbasic euryteles, are abundant on the septae, particularly along the free edge. Numerous microvilli are interspersed among the cilia.

Large, smooth, dome-shaped structures, approximately $3\mu m$ in size, and surrounded by microvilli (Fig. 14), occur on the septal epithelium and inner surface of the oral disc. These structures may represent nematocyst sites or the release of secretory material. Cells of the SPC are capable of ingesting small particles as evidenced by the ingestion of carmine particles which remain in such cells for several days afterwards.

Gastrodermal cells (Fig. 15) which constitute the remainder of the gastrocoel lining possess a single cilium and extremely abundant, long microvilli consistent with their absorptive function.

Ciliary currents

Bright field and phase contrast microscopy reveals that cilia associated with epidermal and gastrodermal cells are highly motile. Motile epidermal cilia generate a vigorous current which flows distally from the pedal disc, along the body column, tentacles and oral disc, carrying small particles away from the organism (Fig. 16). A similar current exists along the gastrodermal epithelium (Fig. 17). Carmine particles falling on the gastric septae and body wall are carried toward the mouth. The current is particularly rapid along the septal edge.

Attempts to reverse the ciliary beat either mechanically or chemically by feeding activators were unsuccessful. The addition of *Artemia* extracts or a 10^{-5} M reduced glutathione solution resulted in a typical feeding response (Loeb & Blanquet, 1973) with no reversal of either external or internal currents. During

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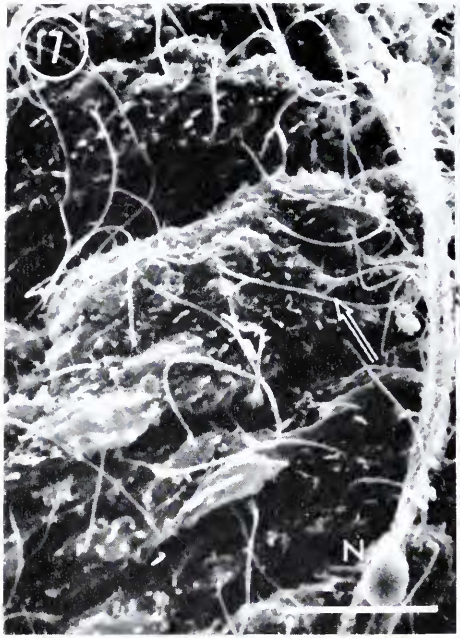
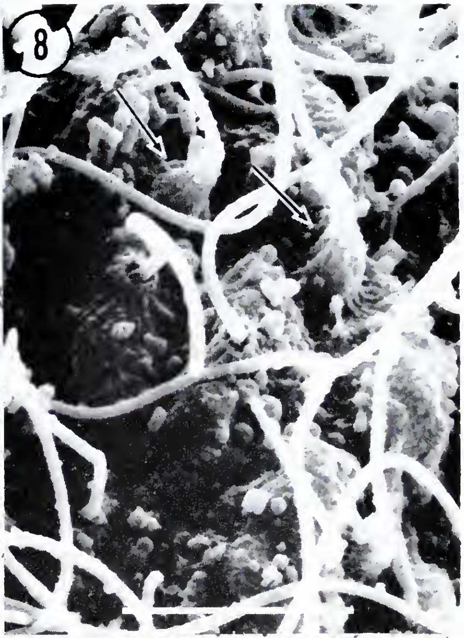
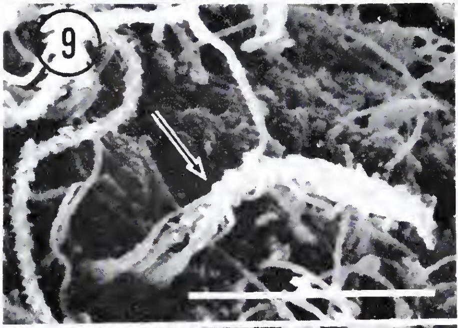
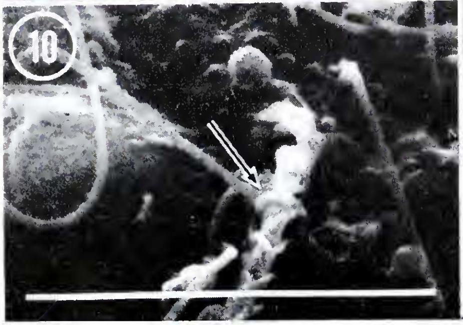
the surface and the fine, filamentous strands which extend between microvilli; 10 μm scale marker.

FIGURE 6. Epithelial surface of the oral disc. Cilia arise from craters formed by a circlet of short, fused microvilli. Few microvilli occur elsewhere on the cell surface; 10 μm scale marker.









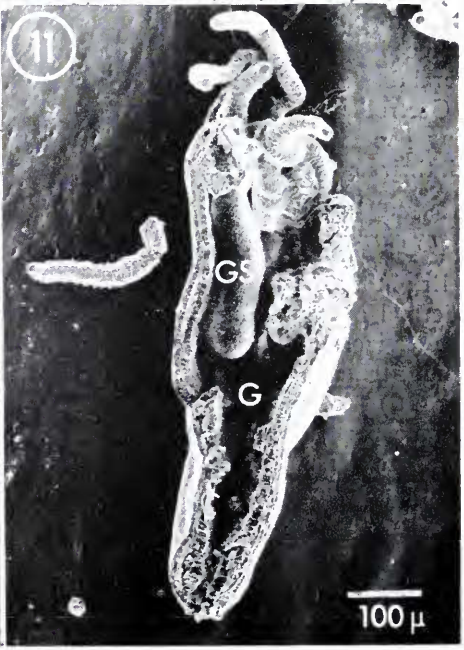


FIGURE 7. Surface of tentacle showing the distribution of cilia and microvilli. A free nematocyst (N, holotrichous isorhiza) with its everted, barbed thread is present. Discharged threads of unliberated nematocysts are also seen. Numerous broad-based cnidocils appear on the tentacle surface (*e.g.*, arrow); 10 μm scale marker.

feeding, the tentacles bend toward the mouth and are frequently inserted into the gastrocoel. In this position, small particles may be swept into the gastrocoel by the current along the tentacles. These are caught almost immediately in the outward flow and are carried away. Thus, in both feeding and non-feeding situations, small particles move away from the polyp.

Discussion

The scyphopolyp, or scyphistoma, represents the asexual reproductive stage in the dimorphic life cycle of many scyphozoan medusae. Such polyps are structurally more complex than hydrozoan polyps but less complex than anthozoan polyps. Compared to the extensive research carried out on hydrozoan and anthozoan polyps, little work has been done on scyphistomae.

Consideration of cell surface interaction with the environment is essential to an understanding of cnidarian function. Scanning electron microscopy (SEM) is ideally suited to survey the surfaces of small organisms. The excellent resolution, increased depth of field and the three dimensional effect of SEM images enables the study of the detailed morphology and distribution of fine surface features. To our knowledge, however, only one survey of both the epidermal and gastrodermal epithelia of a cnidarian polyp has been reported (Beams, Kessel and Shih, 1973). This work concerned the fresh water polyp, *Hydra,* and we believe the present investigation on *Chrysaora* is the first such study on a scyphozoan polyp. SEM enables a better integration of data from light and transmission electron microscopy and allows a more accurate correlation of structure and function. This is of value in comparative studies and in the understanding of the structural adaptation of polyps to different environments.

In marked contrast to *Hydra,* all regions of *Chrysaora* are extensively ciliated. The appearance and arrangement of microvilli around the bases of *Chrysaora* cilia varies; these microvillar configurations being distributed differentially over the polyp. In *Chrysaora,* the tentacles, upper calyx, and especially the scyphopharyngeal-filament complex (SPC) represent the most heavily ciliated regions of the polyp. As these regions are most likely to receive external stimuli, the distribution of cilia is consistent with the possible function of at least some as sensory receptors. A well developed sensory capability would especially benefit an estuarine organism such as *Chrysaora* which is subject to a constantly changing environment.

Sensory cilia have been reported in numerous cnidarians (McConnell, 1932; Mackie, 1960; Burnett and Diehl, 1964; Lentz and Barrnett, 1965; Jha and Mackie, 1967). Josephson (1961) has demonstrated that certain hydroids are

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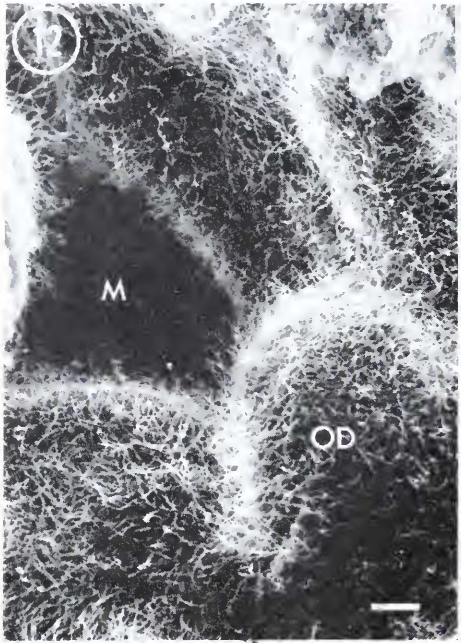
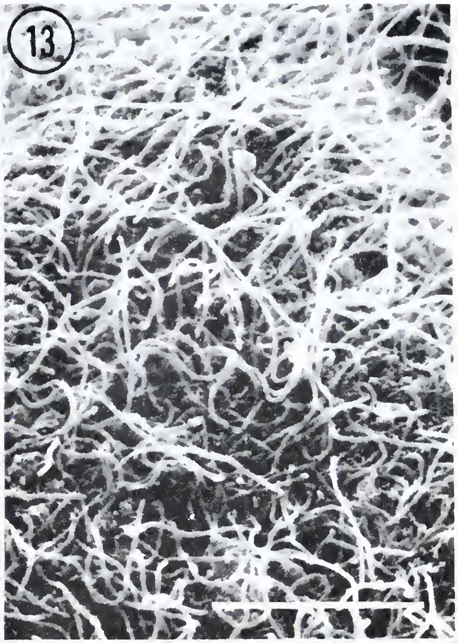
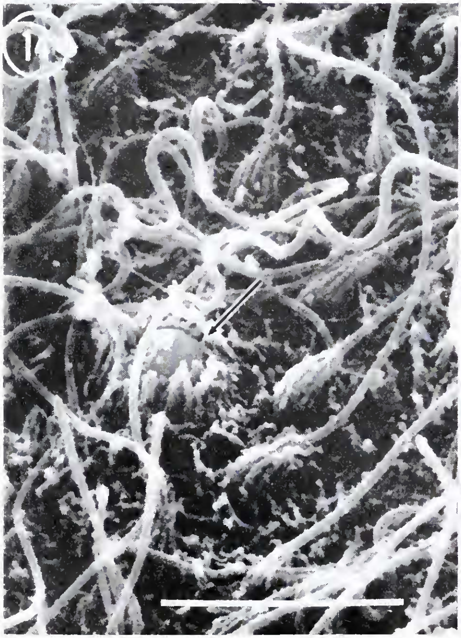
FIGURE 8. Detailed view of the broad conical bases of tentacle cnidocils (arrows). These clearly differ from the circlets of short microvilli surrounding the cilia immediately below them; 10 μm scale marker.

FIGURE 9, 10. Surface of tentacle depicting the close association of broad-based cnidocils with discharged nematocyst threads. Note that the cnidocil shafts are entwined around the discharged threads; 10 μm scale marker.

FIGURE 11. Polyp bisected longitudinally to reveal the gastrocoel (G) and one fold of the prominent septae (GS). The diploblastic nature of the polyp is readily apparent in the wall of the calyx.







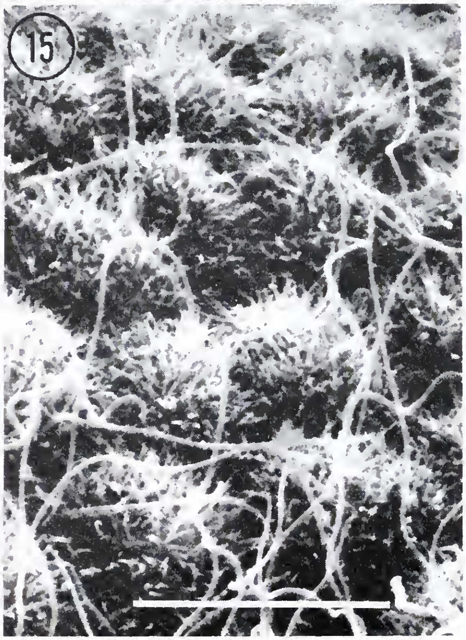


FIGURE 12. Mouth (M) and inner surface of the oral disc (OD)as seen from within the gastrocoel. This is the most heavily ciliated region of the polyp; 10 μm scale marker.

FIGURE 13. Detailed view of the inner surface of the oral disc. Cilia are so numerous they obscure the cell surface; 10 μm scale marker.

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responsive to weak water-borne stimuli. Cilia have been shown to act as receptors for such stimuli in ctenophores and chaetognaths (Horridge, 1965; Horridge and Boulton, 1967). Cilia are also associated with cnidarian statocysts (Horridge, 1969) and photoreceptors (Horridge, 1969; Eakin and Westfall, 1962).

Transmission electron microscopy has shown that cnidocils are modified cilia which arise from a broad base of fused microvilli or sterocilia (Chapman and Tilney, 1959a, b; Lentz, 1966; Westfall, 1965, 1970). The broad-based cilia described in the present study as cnidocils correspond to these descriptions and resemble the cnidocils seen by SEM in *Hydra* (Westfall and Enos, 1972; Beams, Kessel and Shih, 1973). Indeed, the distribution of these broad-based cilia parallels the distribution of nematocysts through the polyp epithelia, and discharged nematocyst threads consistently originate at the bases of these structures. The long cnidocil shafts are often entwined around discharged nematocyst threads (Figs. 9, 10) consistent with the observation of Robson (1953) that nematocyst threads rotate as they discharge.

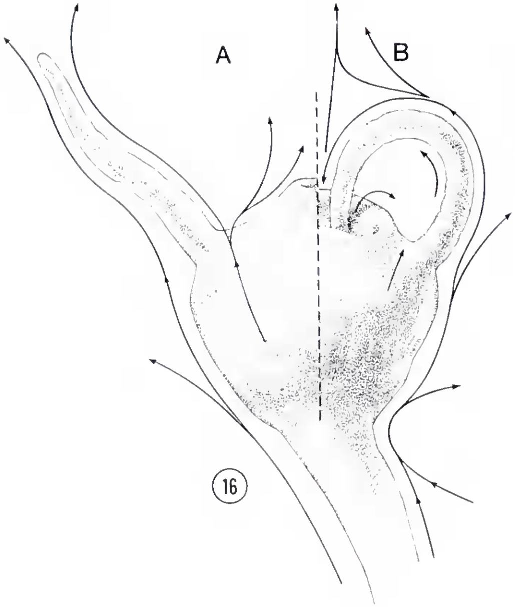
Of special functional significance is the distally directed water currents generated by motile cilia in all regions of the polyp. The speed of these currents appear directly related to the density of cilia in each area. This is most obvious over the heavily ciliated SPC where the current is particularly rapid. Ciliary currents and the increased surface area provided by micromovilli would facilitate the vital diffusion dependent processes of cellular respiration and excretion. The continuous ventilation accomplished by motile cilia, particularly in the gastrocoel, provides a metabolically inexpensive alternative to the periodic ventilation of the organism by body muscle contraction.

As relatively non-motile, estuarine animals, *Chrysaora* polyps are exposed to a constant shower of silt and fine particulate matter brought in by rivers and tributaries. Polyps rapidly succumb when covered by silt and mud (D. Cargo, Cheaspeake Biological Laboratory, Oxford, Maryland, personal communication) and, hence, distally directed ciliary currents would prevent the accumulation of material in the vicinity of the polyp. In addition, such currents could discourage the settling of larvae which compete with the polyp for available space. In contrast, pond-dwelling, freshwater *Hydra* are sparsely ciliated (Beams, Kessel and Shih, 1973).

The well developed septae of *Chrysaora* polyps provide an increased surface area over which digestive processes occur. As *Chrysaora* often captures and ingests relatively large and active prey such as fish fry and crustaceans, the nematocysts of this region may serve to further sting and immobilize such organisms. Although Chapman (1966) feels that the septae serve to accomodate the cord muscles used for polyp contraction, our observations suggest significant, additional roles. Cells of the SPC actively phagocytize small particles from the gastrocoel as demonstrated by the ingestion of carmine and thus it is likely that they ingest food particles as well. Phagocytosis or lysis of bacteria by digestive enzymes may

FIGURE 14. Edge of gastric septum illustrating a large dome-shaped structure surrounded by microvilli. Numerous cnidocils are present; their broad bases consisting of fused microvilli of varying length; 10 μm scale marker.

FIGURE 15. Surface of gastrodermis between the gastric septae. Most cells possess a single cilium and are covered by long microvilli. Microvilli are far more abundant on these absorptive cells than anywhere else on the polyp; 10 μm scale marker.



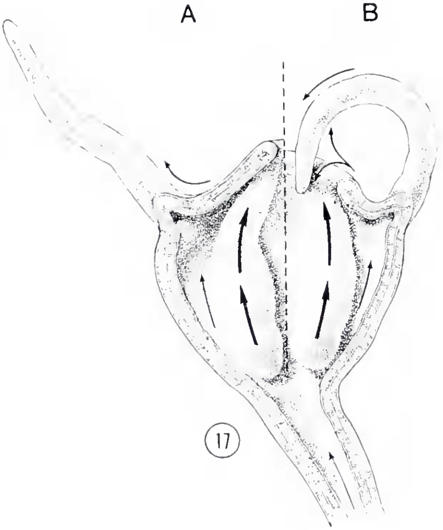


FIGURE 16. Water currents generated by epidermal cilia in non-feeding (A) and feeding (B) polyps.

FIGURE 17. Water currents generated by the gastrodermal cilia in non-feeding (A) and feeding (B) polyps. Currents are particularly rapid along the septal edge.

account for their relative absence on the gastrodermal surface in comparison to the epidermis.

Ciliary currents within the gastrocoel would aid extracellular digestion by mixing ingested food with secreted enzymes. These currents would also increase the number of particle contacts with phagocytic cells. After digestion is complete, the distally directed currents then serve to expel debris and undigested material from the gastrocoel through the open mouth.

The fact that the direction of ciliary currents is unaffected by feeding activators suggests that these currents play little, if any, role in food acquisition. In contrast, other cnidarians such as the sea anemone *Metridium* (Parker and Marks, 1928) demonstrate reversal of ciliary currents which aid in carrying food particles to the mouth.

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SUMMARY

A survey of the epidermal and gastrodermal surfaces of the scyphopolyp *Chrysaora quinquecirrha* by scanning electron microscopy reveals that all epithelia

are ciliated to varying degrees. Cilia are surrounded at their bases by microvilli which vary in configuration. These microvillar configurations are characteristic in different areas of the polyp. Cilia with broad conical bases, corresponding closely in distribution with nematocysts, are thought to represent cnidocils.

Cilia on the epidermal and gastrodermal surfaces are responsible for the generation of a continuous current which flows in an aboral to oral direction in both feeding and non-feeding conditions. The strongest currents are observed along the free edges of the gastric septae, which are the regions of heaviest ciliation in the polyp. Externally, such currents aid in the removal of silt and debris in relatively sessile polyp and serve to circulate gastrovascular fluid and to remove undigested materials.

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LITERATURE CITED

BEAMS, H. W., R. G. KESSEL, AND C. Y. SHIH, 1973. The surface features of *Hydra* as revealed by scanning electron microscopy. *Trans. Amer. Microscop. Soc.*, **92**: 161–175.

BURNETT, A. L., AND N. A. DIEHL, 1964. The nervous system of *Hydra*. I. Types, distribution, and origin of nerve elements. *J. Exp. Zool.*, **157**: 217–226.

CHAPMAN, D. M., 1966. Evolution of the scyphistoma. *Symp. Zool. Soc. London*, **16**: 51–75.

CHAPMAN, G. B., AND L. G. TILNEY, 1959a. Cytological studies of the nematocysts of *Hydra*. I. Desmonemes, isorhizas, cnidocils and supporting structures. *J. Biophys. Biochem. Cytol.*, **5**: 69–78.

CHAPMAN, G. B., AND L. G. TILNEY, 1959b. Cytological studies of the nematocysts of *Hydra*. II. The stenoteles. *J. Biophys. Biochem. Cytol.*, **5**: 79–84.

EAKIN, R. M., AND J. A. WESTFALL, 1962.. Fine structure of photoreceptors in the hydromedusan, *Polyorchis penicillatus*. *Proc. Nat. Acad. Sci.*, **48**: 826–833.

HORRIDGE, G. A., 1965. Non-motile sensory cilia and neuromuscular junctions in a ctenophore independent effector organ. *Proc. Roy. Soc. London Series*, **B162**: 333–350.

HORRIDGE, G. A., 1969. Statocysts of medusae and evolution of stereocilia. *Tissue and Cell*, **1**: 341–353.

HORRIDGE, G. A., AND P. S. BOULTON, 1967. Prey detection by Chaetognatha via a vibration sense. *Proc. R. Soc. London Series*, **B168**: 413–419.

JHA, R. K., AND G. O. MACKIE, 1967. The recognition, distribution and ultrastructure of Hydrozoan nerve nets. *J. Morphol.*, **123**: 43–62.

JOSEPHSON, R. K., 1961. The response of a hydroid to weak water-borne disturbances. *J. Exp. Biol.*, **38**: 17–27.

LENTZ, T. L., 1966. *The Cell Biology of Hydra*. North-Holland Pub. Co., Amsterdam, 199 pp.

LENTZ, T. L., AND R. J. BARRNETT, 1965. Fine structure of the nervous system of hydra. *Amer. Zool.*, **5**: 341–356.

LOEB, M. J., AND R. S. BLANQUET, 1973. Feeding behavior in polyps of the Chesapeake Bay sea nettle, *Chrysaora quinquecirrha* (Desor, 1848). *Biol. Bull.*, **145**: 150–158.

MACKIE, G. O., 1960. Studies on *Physalia physalis* L. Part 2: Behavior and Histology. *Discovery Reports*, **30**: 369–408.

MCCONNELL, C. H., 1932. The development of the ectodermal nerve net in the buds of Hydra. *Quart. J. Microscop. Sci.* **75**: 495–509.

PARKER, G. H., AND A. P. MARKS, 1928. Ciliary reversal in the sea anemone, *Metridium*. *J. Exp. Zool.*, **52**: 1–6.

ROBSON, E. A., 1953. Nematocysts of *Corynactis:* the activity of the filament during discharge. *Quart J. Microscop. Sci.*, **94**: 229–235.

TCHEOU, T. C., 1930. Le cycle evolutif du scyphistome de *Chrysaora*. *Trav. Sta. Biol. Roscoff*, **8**: 1–179.

WEILL, R., 1930. Essai d'une classification des nematocystes des cnidaires. *Bull. Biol. Fr. Belg.*, **64**: 141–156.

WEILL, R., 1934. Contributions a l'etude des cnidaires et de leurs nematocysts. *Trav. Sta. Zool. Wimercux,* **10**: 1–347.

WESTFALL, J. A., 1965. Nematocysts of the sea anemone, *Metridium. Amer. Zool.,* **5**: 377–393.

WESTFALL, J. A., 1970. The nematocyst complex in a Hydromedusan, *Gonionemus vertans. Z. F. Zellforsch Mikrosk. Anat.*, **110**: 457–470.

WESTFALL, J. A., AND P. D. ENOS, 1972. Scanning and electron microscopy of the isolated cells of *Hydra littoralis*. 30th Ann. Proc. Electron Micros. Soc. Amer., pp. 160–161. Los Angeles.